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# Dynamics of Flavonoid Accumulation During the Flowering Phenological Phase of Narrow-Leaf Vetch (*Vicia angustifolia* L.) under Saline Soil Conditions

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In this study, the dynamics of flavonoids during the flowering phenological phase of narrow-leaf vetch (*Vicia angustifolia* L.) grown on weak and moderately saline soils of the Syrdarya region was studied. The content of apigenin, gallic acids, kaempferol, quercetin and rutin was determined in the leaves, stems and roots of this plant using high-performance liquid chromatography (HPLC). Gallic acid levels in narrow-leaf vetch grown in moderately saline soils increased to 173.479 mg/L in the leaves, 157.502 mg/L in the stems, 142.645 mg/L in the roots, and 157.013 mg/L in the flowers. Kaempferol content reached 12.242 mg/L in the leaves and 12.706 mg/L in the flowers, but decreased to 12.245 mg/L in the stems and 12.007 mg/L in the roots. Quercetin increased to 48.623 mg/L in the flowers under moderately saline conditions, was not detected in the roots, decreased to 29.707 mg/L in the leaves, and was absent in the stems under weakly saline soil. Level of increased to 148.286 mg/L in the leaves and 56.728 mg/L in the flowers under moderately saline soil conditions, but decreased to 114.731 mg/L in the stems and 54.975 mg/L in the roots. In conclusion, these changes indicate the role of flavonoids in plant adaptation to salinity stress and open up opportunities for optimizing the cultivation of narrow-leaf vetch as a cover crop in saline areas.
**Keywords:** Adaptation, flavonoids, narrow-leaf vetch, quercetin, salinity stress.

## INTRODUCTION

Flavonoids are characterized as the low molecular weight polyphenolic compounds universally distributed in *planta (*Shomali et al., 2022). Flavonoids are synthesized in specific sites of plant cells, and they control different physiological activities, such as the germination of spores and seeds, the development of aroma and color of flowers, seedlings growth, and also attracting the pollinators toward flower pollen for its dispersion (Griesbach et al., 2005, Panche et al., 2016).

Research (Chutipaijit et al., 2009) has shown that flavonoid accumulation in plants under salt stress conditions may enhance their salt tolerance. Salinity affects nodule formation in legumes by inhibiting signaling between the host plant and its symbiont. However, flavonoids have been shown to enhance symbiont-host signaling, improve nodule formation, and ultimately stimulate plant growth under salt stress (Miransari et al., 2009). Flavonoids also have antioxidant properties; they can neutralize reactive oxygen species (ROS) during stress (Baskar et al., 2018) and limit the metabolic activity of enzymes involved in ROS generation processes, thereby stimulating antioxidant protection. The diversity of flavonoid structures allows them to interact simultaneously with a huge number of biomolecules (Mathesius, 2018) Such as the flavonoid apigenin, kaempferol, quercetin, gallic acid and rutin, exhibit antioxidant properties, modulate reactive oxygen species (ROS) and protect against cell damage (Hernández et al., 2009; Yonekura-Sakakibara et al., 2019). Their accumulation varies with plant age, organ type, and ecological conditions, reflecting adaptive strategies (Pourcel et al., 2007).

Legumes have biological, agronomic and social significance. These crops are the main source of vegetable protein, which are widely used in the food industry, as well as in animal husbandry. After their cultivation, biological nitrogen remains in the soil, which increases soil fertility. They are a

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component in a heterogeneous agrocenosis, where the quality of forage and crop yields increase. Winter and spring varieties of these crops allow you to get two harvests per year, which contributes to the year-round use of soils, increasing the productivity and income of farms (Ismoilova et al., 2023). Abiotic stresses affect different aspects of plants' physiological, biochemical, and molecular status (Austen et al., 2019; Alsobaei, 2024). Salinity is a significant abiotic stress factor (Suprasanna et al., 2016; Muchate et al., 2016) that causes metabolic and physiological disturbances in plants, affecting their development, yield, growth and quality, inhibits plant growth by reducing leaf water potential, causing physiological and morphological changes, generating reactive oxygen species, ion toxicity, increasing osmotic stress and altering biochemical processes (Pandit, 2024). Narrow-leaved vetch (*Vicia angustifolia L.*) belongs to the legume family (Fabaceae) and is an annual plant. This plant is grown as a siderate crop in the fields of the Syrdarya region of the Republic of Uzbekistan. The soils of the Syrdarya region have varying degrees of salinity (weak, medium and strong), which creates difficulties for the cultivation of agricultural crops, but narrow-leaved vetch grows normally in conditions of such salinity. The content of chlorophyll and amino acids in this plant has been studied (Ismoilova et al., 2024), but the dynamics of flavonoids at development stages has not been studied.

Studying the dynamics of flavonoid formation is of both practical and theoretical importance. Theoretically, studying the dynamics is important to determine the biochemical role of flavonoids in plant life (Zaprometov, 1993).

The aim of this study was to determine the content of apigenin, kaempferol, quercetin, gallic acid, and rutin in the flowering phenological phase of narrow-leaf vetch (*Vicia angustifolia L.*) grown in weak and moderately saline conditions of the Syrdarya region, to reveal their role in salt tolerance, and to demonstrate the possibilities of optimizing its cultivation as a siderate crop in saline soils.

## MATERIALS AND METHODS

***Plant material*:** Narrow-leaf vetch (*Vicia angustifolia L.*) was grown in the weakly and moderately saline soil conditions of the Syrdarya region in accordance with the appropriate agrotechnical requirements for conducting research. Samples were taken to determine the amount of flavonoids in the flowering phenological phase.

***Sample preparation*:** Leaves, stems, and roots (1 g each) from 10 plants per age group were extracted with 20 mL of 96% ethanol at 30°C for 75 minutes using a magnetic stirrer, filtered, and stored at 4°C.

***HPLC analysis*:** Flavonoids were quantified using an Agilent HPLC system with a Zorbax guard cartridge (4.6 mm ID × 12.5 mm) and a Perkin Elmer C18 column (250 × 4.6 mm, 5 µm). The mobile phase (0.5% acetic acid in water [A] and acetonitrile [B]) followed a gradient: 60% A/40% B (1 min), 70% A/30% B (3 min), 55% A/45% B (6 min), 80% A/20% B (10 min), stop (12 min), at 0.8 mL/min and 40°C. Detection occurred at 254 nm, with standards at 0.025 and 0.05 mg/mL.

***Statistical analysis*:** Data were analyzed using ANOVA and Tukey's test ($p < 0.05$), reported as means ± SD.

## RESULTS

Flavonoid levels varied significantly across organs (Table 1).

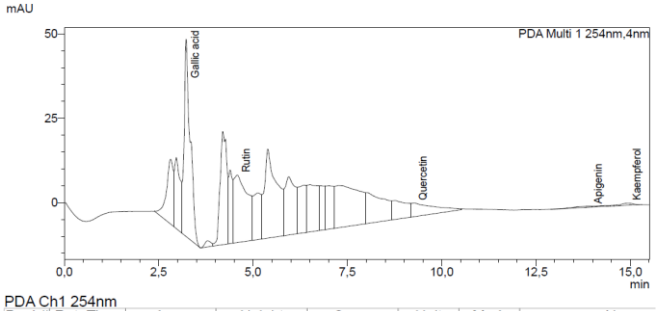
***Chromatographic profiles*:** Chromatograms (Figures 1-8) detailed flavonoid distribution: Narrow-leaved vetch (*Vicia angustifolia L.*) leaves chromatogram grown in weak saline soil (Figure-1) showed peaks at 254 nm: rutin (147.480 mg/L, retention time (RT) ~6 min), gallic acid (165.798 mg/L, RT ~3 min), kaempferol (13.203 mg/L, RT ~15 min), apigenin (14.503 mg/L, RT ~14 min), and quercetin (45.928 mg/L, RT ~9 min), with gallic acid peaking. Narrow-leaved vetch (*Vicia angustifolia L.*) leaves chromatogram grown in moderately saline soil (Figure-2) showed peaks at 254 nm: rutin (148.286 mg/L, retention time (RT) ~5 min), gallic acid (173.479 mg/L, RT ~3 min), kaempferol (12.242 mg/L, RT ~15 min), quercetin (29.707 mg/L, RT ~10 min), with no apigenin, reflecting a shift in flavonoid priorities.

Narrow-leaved vetch (*Vicia angustifolia L.*) stemes chromatogram grown in weak saline soil (Figure 3) showed peaks at 254 nm: rutin (133.502 mg/L, retention time (RT) ~4 min), gallic acid (148.574 mg/L, RT ~3 min), kaempferol (12.568 mg/L, RT ~15 min), apigenin and quercetin are no detected. Narrow-leaved vetch stemes chromatogram grown

**Table 1. Flavonoid content (mg/L) in narrow-leaf vetch (*Vicia angustifolia* L.) organs during early development in saline soils.**

| Organ | Soil environment | Amount of flavanoids, mg/ml | | | | |
|---|---|---|---|---|---|---|
| | | Apigenin | Gallic Acid | Kayempferol | Quercetin | Rutin |
| Leaves | Weak | 14.503±0.8 | 165.798±3.4 | 13.203±0.7 | 45.928±1.5 | 147.480±3.8 |
| | Moderately | ND | 173.479±4.2 | 12.242±0.6 | 29.707±0.8 | 148.286±4.2 |
| Stems | Weak | ND | 148.574±3.6 | 12.568±0.4 | ND | 133.502±3.0 |
| | Moderately | ND | 157.502±3.6 | 12.245±0.5 | 27.906±0.9 | 114.731±2.8 |
| Roots | Weak | ND | 140.777±3.2 | 12.258±0.7 | 27.883±0.8 | 61.205±2.2 |
| | Moderately | ND | 142.645±2.8 | 12.007±0.5 | ND | 54.975±1.7 |
| Floral part | Weak | ND | 156.346±3.7 | 12.540±0.3 | 42.550±1.4 | 55.326±1.9 |
| | Moderately | ND | 157.013±3.5 | 12.706±0.6 | 48.623±1.2 | 56.728±2.2 |

in moderately saline (Figure-4) showed peaks at 254 nm: rutin (114.731 mg/L, retention time (RT) ~4 min), gallic acid (157.502 mg/L, RT ~3 min), kaempferol (12.245 mg/L, RT ~15 min), quercetin (27.206 mg/L, RT ~9 min), apigenin is no detected. Narrow-leaved vetch roots chromatogram grown in weak soil (Figure-5) showed peaks at 254 nm: rutin (61.205 mg/L, retention time (RT) ~4 min), gallic acid (140.777 mg/L, RT ~3 min), kaempferol (12.258 mg/L, RT ~15 min), quercetin (27.883 mg/L, RT ~11 min), apigenin is no detected.



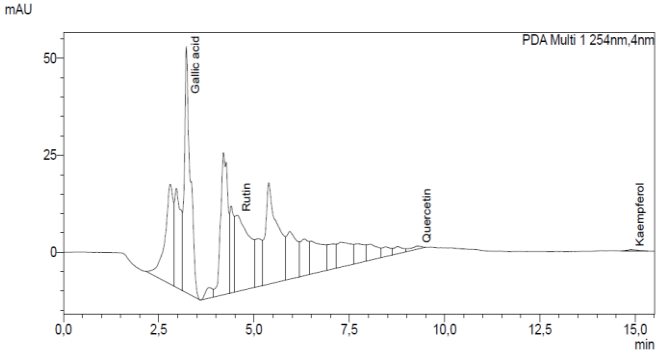

PDA Ch1 254nm

| Peak# | Ret. Time | Area | Height | Conc. | Unit | Mark | Name |
|---|---|---|---|---|---|---|---|
| 3 | 3,226 | 642675 | 58442 | 165,798 | mg/L | V | Gallic acid |
| 7 | 4,581 | 505815 | 19986 | 147,480 | mg/L | V | Rutin |
| 18 | 9,269 | 164075 | 4049 | 45,928 | mg/L | V | Quercetin |
| 19 | 13,909 | 12237 | 288 | 14,503 | mg/L | | Apigenin |
| 22 | 14,932 | 13785 | 594 | 13,203 | mg/L | V | Kaempferol |
| Total | | 1338588 | 83358 | | | | |

**Figure 1. Chromatographic indicators of the content of gallic acids, rutin, quercetin, apigenin and kaempferol in the leaves of narrow-leaf vetch (*Vicia angustifolia* L.) at the flowering phenological phase in weakly saline soil (Mobile phase speed 1 ml/min. Detection wavelength 254 nm).**

Narrow-leaved vetch (*Vicia angustifolia L.*) roots chromatogram grown in moderately saline soil (Figure-6) showed peaks at 254 nm: rutin (54.975 mg/L, retention time (RT) ~4 min), gallic acid (142.645 mg/L, RT ~3 min), kaempferol (12.007 mg/L, RT ~15 min), quercetin and. Apigenin are no detected, reflecting a shift in flavonoid priorities. Narrow-leaved vetch flowers chromatogram grown in weak saline soil (Figure-7) showed peaks at 254 nm: rutin (60.863 mg/L, retention time (RT) ~5 min), gallic acid (148.695 mg/L, RT ~3 min), kaempferol (12.032 mg/L, RT ~15 min), apigenin (20.458 mg/L, RT ~11 min), quercetin is no detected, low amount of kaempferol. Narrow-leaved vetch flowers chromatogram grown in moderately saline soil (Figure-8) showed peaks at 254 nm: rutin (55.326 mg/L, retention time (RT) ~5 min), gallic acid (156.346 mg/L, RT ~3 min), quercetin (42.550 mg/L, RT ~10 min), kaempferol (12.540 mg/L, RT ~15 min), apigenin is no detected, the predominance of gallic acids.

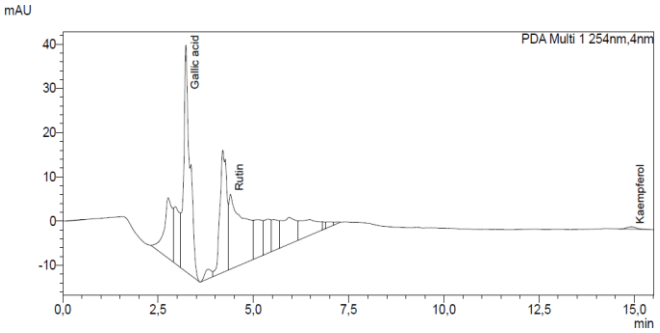
These profiles confirm the flavonoid dynamics associated with increasing soil saline in the organs under salinity stress.




PDA Ch1 254nm

| Peak# | Ret. Time | Area | Height | Conc. | Unit | Mark | Name |
|---|---|---|---|---|---|---|---|
| 3 | 3,223 | 673517 | 63413 | 173,479 | mg/L | V | Gallic acid |
| 7 | 4,561 | 508910 | 19631 | 148,286 | mg/L | V | Rutin |
| 19 | 9,294 | 17868 | 783 | 29,707 | mg/L | V | Quercetin |
| 20 | 14,885 | 6229 | 338 | 12,242 | mg/L | S | Kaempferol |
| Total | | 1206523 | 84165 | | | | |

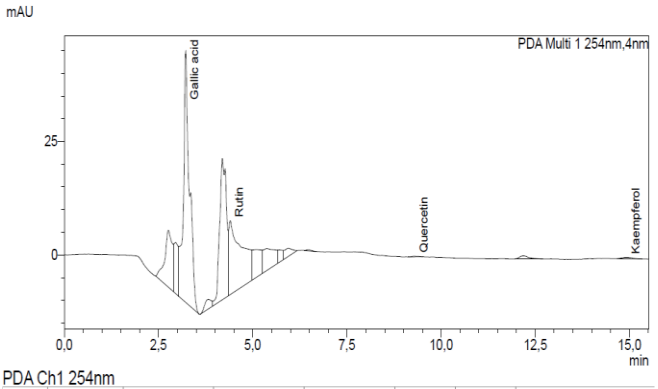
**Figure 2. Chromatographic indicators of the content of gallic acids, rutin, quercetin, apigenin and kaempferol in the leaves of narrow-leaf vetch (*Vicia angustifolia* L.) at the flowering phenological phase in moderately salty soil (Mobile phase speed 1 ml/min. Detection wavelength 254 nm).**




PDA Ch1 254nm

| Peak# | Ret. Time | Area | Height | Conc. | Unit | Mark | Name |
|---|---|---|---|---|---|---|---|
| 4 | 3,226 | 573517 | 51164 | 148,574 | mg/L | V | Gallic acid |
| 7 | 4,397 | 452120 | 16928 | 133,502 | mg/L | V | Rutin |
| 16 | 14,910 | 8790 | 504 | 12,568 | mg/L | | Kaempferol |
| Total | | 1034427 | 68596 | | | | |

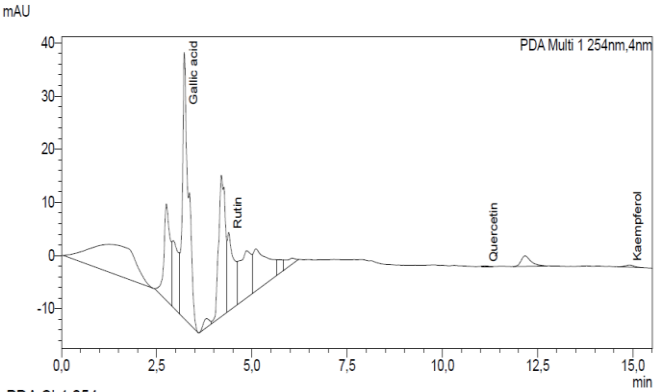
**Figure 3. Chromatographic indicators of the content of gallic acids, rutin, quercetin, apigenin and kaempferol in the stem of narrow-leaf vetch (*Vicia angustifolia* L.) at the flowering phenological phase in weakly saline soil (Mobile phase speed 1 ml/min. Detection wavelength 254 nm).**




PDA Ch1 254nm

| Peak# | Ret. Time | Area | Height | Conc. | Unit | Mark | Name |
|---|---|---|---|---|---|---|---|
| 3 | 3,219 | 609366 | 55467 | 157,502 | mg/L | V | Gallic acid |
| 6 | 4,403 | 380011 | 16300 | 114,731 | mg/L | V | Rutin |
| 12 | 9,308 | 1629 | 145 | 27,906 | mg/L | | Quercetin |
| 14 | 14,924 | 6249 | 344 | 12,245 | mg/L | | Kaempferol |
| Total | | 997255 | 72257 | | | | |

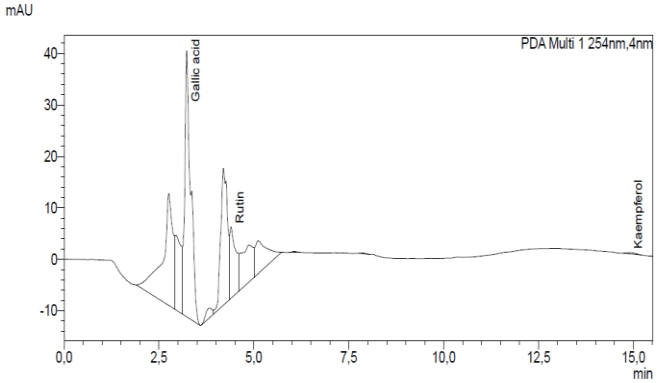
**Figure 4. Chromatographic indicators of the content of gallic acids, rutin, quercetin, apigenin and kaempferol in the stem of narrow-leaf vetch (*Vicia angustifolia* L.) at the flowering phenological phase in moderately salty soil (Mobile phase speed 1 ml/min. Detection wavelength 254 nm).**




PDA Ch1 254nm

| Peak# | Ret. Time | Area | Height | Conc. | Unit | Mark | Name |
|---|---|---|---|---|---|---|---|
| 4 | 3,225 | 542211 | 50139 | 140,777 | mg/L | V | Gallic acid |
| 7 | 4,389 | 174389 | 14793 | 61,205 | mg/L | V | Rutin |
| 12 | 11,107 | 1420 | 139 | 27,883 | mg/L | V | Quercetin |
| 14 | 14,881 | 6354 | 388 | 12,258 | mg/L | | Kaempferol |
| Total | | 724374 | 65459 | | | | |

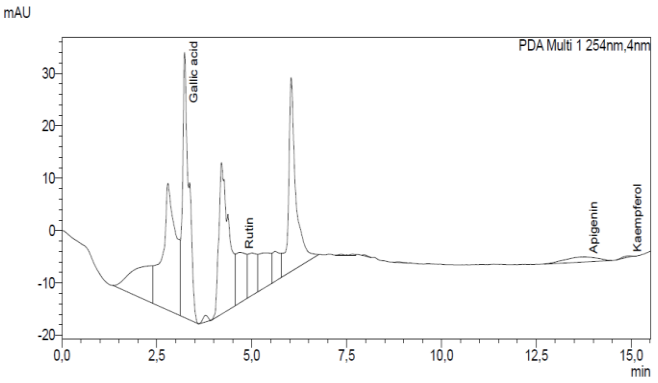
**Figure 5. Chromatographic indicators of the content of gallic acids, rutin, quercetin, apigenin and kaempferol in the root of narrow-leaf vetch (*Vicia angustifolia* L.) at the flowering phenological phase in weakly saline soil (Mobile phase speed 1 ml/min. Detection wavelength 254 nm).**




PDA Ch1 254nm

| Peak# | Ret. Time | Area | Height | Conc. | Unit | Mark | Name |
|---|---|---|---|---|---|---|---|
| 3 | 3,228 | 549709 | 51688 | 142,645 | mg/L | V | Gallic acid |
| 6 | 4,397 | 150457 | 13861 | 54,975 | mg/L | V | Rutin |
| 11 | 14,887 | 4379 | 257 | 12,007 | mg/L | | Kaempferol |
| Total | | 704545 | 65807 | | | | |

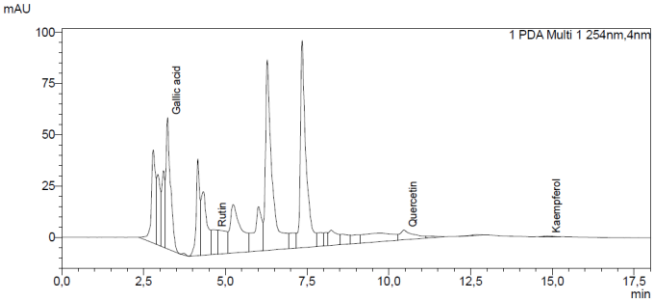
**Figure 6. Chromatographic indicators of the content of gallic acids, rutin, quercetin, apigenin and kaempferol in the root of narrow-leaf vetch (*Vicia angustifolia* L.) at the flowering phenological phase in moderately salty soil (Mobile phase speed 1 ml/min. Detection wavelength 254 nm).**




PDA Ch1 254nm

| Peak# | Ret. Time | Area | Height | Conc. | Unit | Mark | Name |
|---|---|---|---|---|---|---|---|
| 3 | 3,230 | 574001 | 50618 | 148,695 | mg/L | V | Gallic acid |
| 6 | 4,701 | 173076 | 9575 | 60,863 | mg/L | V | Rutin |
| 15 | 13,770 | 57190 | 962 | 20,458 | mg/L | V | Apigenin |
| 16 | 14,924 | 4574 | 263 | 12,032 | mg/L | | Kaempferol |
| Total | | 808842 | 61418 | | | | |

**Figure 7. Chromatographic determination of gallic acids, rutin, quercetin, apigenin and kaempferol in flowers of narrow-leaved vetch (*Vicia angustifolia L.*) in weakly saline soil (Mobile phase speed 1 ml/min. Detection wavelength 254 nm).**




PDA Ch1 254nm

| Peak# | Ret. Time | Area | Height | Conc. | Unit | Mark | Name |
|---|---|---|---|---|---|---|---|
| 4 | 3,228 | 604724 | 63974 | 156,346 | mg/L | V | Gallic acid |
| 8 | 4,626 | 151806 | 12040 | 55,326 | mg/L | V | Rutin |
| 21 | 10,462 | 133630 | 4707 | 42,550 | mg/L | V | Quercetin |
| 24 | 14,845 | 8566 | 474 | 12,540 | mg/L | | Kaempferol |
| Total | | 898726 | 81195 | | | | |

**Figure 8. Chromatographic determination of gallic acids, rutin, quercetin, apigenin and kaempferol in flowers of narrow-leaved vetch (*Vicia angustifolia L.)* in moderately saline soil (Mobile phase speed 1 ml/min. Detection wavelength 254 nm).**

## DISCUSSION

The profiles summarized in Table 1 confirm the flavonoid dynamics associated with increasing soil saline in the organs under salinity stress. These trends and variations are previously reported in various plant speciase, such as Phaseolus vulgaris (Yildiztugay et al., 2020) and also this tresn in mentioned in a review work of Shomali et al. (2022). The highest and lowest phenol and flavonoid content was obtained at the flowering stage of the tropical habitat and the emergence stage of the stem of the cold region (Baghaeifar et al., 2023). According to Afzali et al. (2007), in salinity stress, the dry weight of flowers increased with increasing, while this level decreased the dry weight of flowers for chamomile plant. In present study and based on data presented in result section, with increasing soil salinity, high levels of gallic acid in the leaves (165.798 mg/L, RT ~3 min), stems (157.502 mg/L, RT ~3 min), roots (142.645 mg/L, RT ~3 min), and flowers (157.013 mg/L, RT ~3 min) of narrow-leaved Vetch (*Vicia angustifolia L.*) indicate antioxidant activity against reactive oxygen species (ROS) generated by increasing salinity (Kreft et al., 2003). The decrease in kaempferol to 12.242 mg/L in leaves, 12.245 mg/L in stems, 12.007 mg/L in roots, and an increase to 12.706 mg/L in flowers indicate a temporary photoprotective function. The increase in the highest level of quercetin in flowers to 48.623 mg/L supports a role in flower development under stress. The increase in apigenin to 145.432 mg/L in leaves and rutin to 148.286 mg/L in leaves is consistent with their role in protecting against ultraviolet radiation (Salehi et al., 2019). These patterns reflect salinity-driven metabolic adjustments, mediated by the phenylpropanoid pathway (Sato & Sankawa, 1983).

Flavonoids play a protective role by scavenging free radicals before they can damage cells under stress conditions. They are classified as important secondary metabolites synthesized in almost every part of the plant during plant interactions with the environment. Due to the important role of flavonoids in plant physiological and biochemical responses in healthy and stressed environments, future research should be aimed at identifying metabolic, molecular, and signaling regulators involved in environmental stress tolerance. Also, studying the relationship between flavonoids and plant stress tolerance will help to better understand the mechanisms for plant productivity and sustainable agricultural practices.

***Conclusion*:** In narrow-leaved vetch (*Vicia angustifolia L.*), flavonoids show dynamic shifts under saline soil conditions: in moderately saline soil, gallic acids increase by 7.681 mg/L and rutin by 0.806 mg/L in leaves compared to weakly saline soil, while kaempferol decreases by 0.961 mg/L and quercetin by 16.221 mg/L. In the stem, gallic acids increase by 8.928 mg/L, quercetin is produced by 27.707 mg/L, kaempferol decreases by 0.323 mg/L and rutin by 18.771 mg/L. In the root, the amount of gallic acids increases by 1.868 mg/L, kaempferol decreases by 0.251 mg/L, rutin by 6.23 mg/L, quercetin is not detected. In the flower, gallic acids increase by 0.667 mg/L, rutin by 1.402 mg/L, kaempferol by 0.166 mg/L, quercetin by 6.073 mg/L. Apigenin is detected in the leaves of the plant at 14.503 mg/L under weakly saline soil conditions, and is not recorded in the remaining variants. These changes emphasize the adaptive role of flavonoids in salinity tolerance and provide insights for optimizing the cultivation of narrow-leaved Vetch (*Vicia angustifolia L.*) in saline areas.

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***Conflict of interest*:** The authors declare no conflicts of interest related to this report.

***Availability of data*:** Data supporting the findings of this study are available from the corresponding author upon reasonable request.

***Consent to participate*:** Not applicable.

***Informed consent*:** N/A

***Consent for publication*:** All authors have read and approved the final manuscript for publication

***SDGs addressed*:** Zero hunger; climate action; life on land.

***Policy referred*:** Agricultural and environmental policy related to salinity management and crop sustainability.

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